PDBP
Parkinson's Disease
Biomarkers Program

Isolation of Peripheral Blood Mononuclear Cells

CLIMB12new:PBMC

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1. CLIMB12new: Isolation of Peripheral Blood Monoculear Cells

All blood samples must be considered biohazards and all procedures must be done according to current OSHA guidelines. Always wear gloves and personal protective equipment (PPE) when handling blood specimens. All work needs to be performed in a tissue culture hood using proper sterile technique if you are isolating peripheral blood mononuclear cells (PBMCs) for freezing or use in immunological assays.

1.1. Blood Collection

1. Draw blood into plastic green-top vacutainer tubes containing Lithium Heparin using a 21-gauge butterfly needle and gently mix by inverting the tube 5-10 times.

2. Keep tubes at room temperature until transport to the lab. The tubes should be sent with the next available blood runner for immediate processing in the lab.

1.2. Isolation of Peripheral Blood Mononuclear Cells (PBMCs) – CLIMB 1 only

This protocol describes the procedure for processing four green-top tubes. Please scale up or down the amount of tubes and reagents if you start with a different volume of blood.

1. Remove Flash Freeze (20% DMSO, 80% FBS) and 100% FBS from the +4ºC fridge and leave at room temperature to warm up.

2. Pour blood from the green-top tubes into two 50 ml Falcon tubes. Rinse all green-top tubes with HBSS (7 ml to each tube) and add the blood/HBSS mixture to the Falcon tubes. Make sure to keep equal volume between the two Falcon tubes.

3. Mix Ficoll solution by inverting the bottle several times. Fill a 10 ml pipette up to the red line (roughly 13 ml) and insert to the bottom of one of the blood containing Falcon tubes. Carefully remove the pipette from the pipet-aid and let the Ficoll drain into the tube. When the Ficoll level has equilibrated with the level of the blood, gently lift the pipette to the top of the Ficoll layer to drain the remaining Ficoll from the pipette (you will not be able to totally empty the pipette). Cap the pipette with your finger and slowly remove the pipette from the Falcon tube without disturbing the Ficoll/blood interface. If done correctly, the Ficoll should remain as a clear layer on the bottom of the tube with the blood as a separate layer on top. Repeat with the second Falcon tube.

4. Alternatively, you may overlay the blood on top of the Ficoll instead of underlying the Ficoll under the blood as described above. Add 13 ml of Ficoll each to two clean 50 ml Falcon tubes. Use a 25 ml pipette to collect all blood/HBSS mixture and carefully overlay on top of the Ficoll. The can be done by holding the Falcon tube containing the Ficoll almost parallel to the surface of the hood and slowly letting the blood out of the pipette.

Note: Be careful not to disturb the Ficoll layer or the separation of the PBMCs will not work properly.

5. Immediately spin at 800*g* (2000 rpm in our large refrigerated centrifuges) for 30 min at room temperature with the **BRAKE OFF.**

   After centrifugation, the sample should have separated into four layers: 1. a pinkish layer containing plasma and platelets on top; 2. the "buffy coat" containing PBMCs as a whitish fluffy layer just under the plasma; 3. the Ficoll as a clear layer between the buffy coat and the red blood cells; and 4. granulocytes and red blood cells at the bottom.

6. As soon as the centrifuge stops, remove and discard the upper plasma layer by pipetting. Carefully collect the buffy coat containing the PBMCs with a clean pipette and transfer to a clean 50 ml Falcon tube. It is critical to remove the entire buffy coat, without including any Ficoll or plasma, to avoid contamination with granulocytes or plasma proteins.

7. Wash PBMCs by adding HBSS to the 45 ml line and spin at 300*g* (1300 rpm in our centrifuges) for 5 min at room temperature.

8. Pour off the HBSS without disturbing the PBMC pellet. Resuspend cells in 10 ml HBSS and remove enough cells for counting (usually 10 µl; see separate protocol for cell counting). Add HBSS up to 45 ml line and spin at 300*g* (1300 rpm in our centrifuges) for 5 min at room temperature. Count cells during the spin. Cells are now ready for freezing (see separate protocol for cryopreservation) or use in immunological assays.

1.3. Supplies

Product	Vendor	Cat#
BD Vacutainer Plasma Tubes (green top), 10 ml, plastic, with Lithium Heparin	Fischer Scientific	02-689-7
21G Winged Blood Collection Sets	Fischer Scientific	22-024-821
Disposable Tourniquets	Fischer Scientific	22-035-365
Alcohol Pads	Fischer Scientific	06-669-62
Gauze Pads	Fischer Scientific	22-415-468
Bandaids (plain)	Fischer Scientific	17-442-3C
Bandaids (Loony Tunes)	Fischer Scientific	19-027-304A
Absorbent Underpads (blue diapers)	Owens and Minor	39829
Sterile 1000 µl Tips (Corning International)	Fischer Scientific	07-200-304
Cryovials, 1 ml	Fischer Scientific	12-565-164N
Cryocaps (assorted colors)	Fischer Scientific	12-565-180
Cryogenic Storage Labels	Fischer Scientific	LCRY1700
Fiberboard Freezer Boxes	Fischer Scientific	11-678-24A
Box Dividers (9x9 grid)	Fischer Scientific	13-989-218
Tape (assorted colors)	Fischer Scientific	11-880-5R
Hank's Balanced Salt Solution (HBSS), BioWhittaker	Fischer Scientific	BW10-508F
Ficoll-Paque PLUS	GE Healthcare	17-1440-03
Falcon Tubes, 50 ml	Fischer Scientific	05-583-60
Serological Pipettes, 10 ml, sterile	Fischer Scientific	13-675-20
Serological Pipettes, 25 ml, sterile	Fischer Scientific	13-675-30